Abstract

This work studies the "O" subgroup of FOX transcription factors, which consists of four members (FOXO1, FOXO3, FOXO4 and FOXO6). They are important regulatory molecules that play a critical role in a number of physiological and pathological processes such as cell cycle control, the body's response to stress, differentiation and apoptosis. Due to their ability to induce cell death, they are generally considered to be tumor suppressors. However, recent studies have shown that they can also induce an opposite effect, i.e. to promote tumor progression or induce resistance to drugs used in the therapy of certain types of tumors. Despite intensive research, a number of questions regarding the function of FOXO proteins still remain unanswered. One question is whether the small structural differences observed in the highly conserved DNA-binding domains (DBD) of FOXO transcription factors affect their DNA-binding affinities. Furthermore, it is unclear whether the recently described protein-protein interaction of FOXO-DBD with the transcription factor p53 affects their DNA-binding affinity. Moreover, the role of the binding site for Mg^{2+} ion which was found in the crystal structure of FOXO4-DBD:DNA, is also still not understood. To clarify these questions, the DNA-binding domains of the human transcription factors FOXO3 and FOXO4 (FOXO3-$DBD_{142-267}$ and FOXO4-DBD_{82-207}) were expressed and purified. Next, the DNA-binding affinities of prepared FOXO3-$DBD_{142-267}$ and FOXO4-DBD_{82-207} under different conditions, i.e. in the presence and absence of $p53_{1-312}$ protein, Ca^{2+} and Mg^{2+} ions, were determined using steady-state fluorescence anisotropy measurements. The results showed that the presence of Ca^{2+} ions and $p53_{1-312}$ protein significantly increased the DNA-binding affinities of both FOXO-DBDs. Surprisingly, the presence of Mg^{2+} ions reduced the DNA-binding affinity of both FOXO-DBDs.

Keywords: FOXO, forkhead, p53, transcription factor, protein-protein interaction

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